Measuring Compartment Size and Gas Solubility in Marine Mammals

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LONG-TERM GOALS

The long term goal of this study is to develop methods to estimate marine mammal tissue compartment sizes, and tissue gas solubility. We aim to improve the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of their different morphological and physiological adaptations. The study will also develop a method that enables the determination of gas solubility in different tissue compartments.

OBJECTIVES

This study include two main objectives: to study the morphometrics of marine mammal body compartments and the solubility coefficient for O_2, CO_2 and N_2 of these compartments. Both objectives need the development of new methods to reach their respective goals.

The first objective is aimed at improving the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of the various morphological and physiological adaptations that exist among marine mammals. Previous efforts have been focused on measuring the major O_2 stores, such as muscle mass and myoglobin (Mb) concentration, or total blood volume and hemoglobin content [1]. There is also little or no information for certain tissue compartments such as skin, blubber, muscle, heart, lung, liver, kidneys, spleen or bone. The relative size of each compartment has not been properly calculated with a consistent methodology.

Therefore there is a need to consistently measure the relative size of the different tissues: such as skin, muscle, blubber, heart, and lungs in as many species as possible.

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The second objective is aimed at developing a method that enables the determination of gas solubility in different body compartments. There are limited data on gas solubility in marine mammal tissues: species differences have been found, and variations compared to land mammals are expected [2]. We aim to modify the method developed by Koopman et al. [2] that enables the determination of gas solubility from "solid" tissues (skin, blubber, muscle, brain, liver, kidney). We also aim to study the solubility of all gases (N_2, O_2, CO_2 and H_2) that are routinely found in bubbles of stranded marine mammals [3,4]. This modification will consist of an adaptation of the Scholander method for measuring tissue gas content [5]. Therefore we were hoping to be able to analyze the gas composition of tissues of marine mammals. Within this project, we are expecting to determine the "original" gas composition of the tissues of a given animal and we will analyze the relationship between tissue gas content to amount and composition of gas bubbles found in different tissues by using the methods developed by Bernaldo de Quirós et al. [6,4], in addition to gas solubility studies.

APPROACH

OBJECTIVE 1
Aim 1: Obtaining morphometric data for different species. For this aim fresh specimens of adult animals will be requested from different locations: North Carolina, Cape Cod Bay and from the Canary Islands. In addition, access to bycaught animals will be facilitated by NOAA. A mass dissection protocol to systematically separate the body into discrete anatomical components will be developed in collaboration with Bill McLellan and Ann Pabst, based on their previous experience [7]. Tissues will be weighed separately. Volume will be measured by water displacement. Density will be calculated by dividing the weight by the volume. Finally, we will report the mass of each body compartment as a percentage of the total body mass [8].

Aim 2: Muscle myoglobin determination. Myoglobin content will be calculated for the different muscle groups, including heart, of each specimen following the method described by Polasek and Davis [9]. Dr. Pabst will introduce Dr. Bernaldo de Quiros to this technique.

OBJECTIVE 2
Aim 1: Design of an anaerobic tissue grinder. In 1942, Scholander designed a device for the determination of the gas content in tissues [5]. We will modify this device to allow quick removal of the tissue. In this way, removal and grinding of the tissue will be done as with as little atmospheric air contamination as possible. We will design the device in such way that blood, water and other liquids will be separated from the tissue. The ground tissue will be transferred to an evacuated glass tube.

Aim 2: Determine the tissue solubility coefficient of gases. We will follow the procedure outline in Koopman et al. [2]; although some modifications will be needed in order to study other tissues than blubber and other gases than N_2. The development of this method will be done in collaboration with Dr. Sylva and Dr. Seewald from Woods Hole Oceanographic Institution (WHOI) and Dr. González Díaz from the University of Las Palmas de Gran Canaria (ULPGC). The method will be applied at WHOI and in the Canaries (at the ULPGC). We will validate our method by running samples for which solubility coefficients have been previously reported such as water and olive oil [10]. Once we know the method is generating accurate results, we will determine the solubility coefficient of the gases of interest in the tissues for which morphometrics are measured. We are aware of the complexity of this aim and therefore plan more than one year to develop these techniques and complete this aim. The approach for this aim has changed. See work completed.

Aim 3: Analyzing gas content in tissues. Once tissues have been transferred anaerobically, we will analyze the gas content using the headspace method. The headspace is the vapor in equilibrium with its liquid phase. When a dissolved substance is sufficiently volatile, the determination of its concentration in the vapor phase can be used as a measure of the concentration in the liquid phase if the solubility coefficient is previously known, providing that equilibrium between the vapor and liquid phases has been reached. We aim to determine the gas tension of the different tissues in marine mammals and how it relates to gas composition in the bubbles [4]. The approach for this aim has changed. See results.

WORK COMPLETED

OBJECTIVE 1

The McLellan et al. (2002) mass dissection protocol has been implemented to fullfil the goals of this project and a collaboration with the Pabst lab (UNCW) has been established to collect the data in the same manner so results can be compared.

The number of strandings on Cape Cod has been unusually low compared to previous years, and the NOAA fisheries observers have brought ashore only a few animals. Therefore although our target were adults and robust animals, we dissected every animal that was with decomposition code 2 regardless its age or body condition. Mass dissection data were collected from one spotted dolphin, one bottlenose dolphin, three common dolphins, one elephant seal, three californian sea lions and two elephant seals. Muscle samples (to determine myoglobin content) and tissue samples from various organs (to determine gas solubility in this tissues) of these animals were also collected (Table 1).

Table-1: Animals studied for body size compartments

Specie	ID	Source	Age	Sex	Body condition	Weigh (g)	Lenght (cm)
P.phocoena	IFAW14-034Pp	Cape Cod	subadult	Female	skinny	-	
D.delphis	IFAW14-044Dd	Cape Cod	Subadult	Male	Robust	68800	176
D.delphis	IFAW14-116Dd	Cape Cod	Adult	Male	Fair/Good	94300	
D.delphis	IFAW14-134Dd	Cape Cod	Subadult	Male	Robust	134000	176
S. Frontalis	WAM689	Wilmington	Subadult?	Female	Good	67000	180.5
T.truncatus	WAM690	Wilmington	Subadult?	Male	Fair	89000	195
M. angustirostris	ES3607 (Lucky duck)	California	Calve	Male	Cachexic	33500	121
Z. Californianus	CSL10941 (Mohawk)	California	Adult	Male	skinny	111500	197
Z. Californianus	CSL10972	California	Adult	Female	skinny	62500	151
Z. Californianus	CSL10466	California	Adult	Female	skinny	71500	160.5
H. grypus	DO9587	Cape Cod	Subadult	Female	Robust	36000	104
H. grypus	IFAW14-114Hg	Cape Cod	Juvenile	Female	Robust	25000	99

OBJECTIVE 2

Aim 1: Design of anaerobic grinder. We designed an anaerobic tissue grinder that separates the blood from the tissues. The device was tested in three animals: fresh animal with no bubbles, frozen animal with no bubbles, fresh animal with bubbles.

Aim 2: Determine the tissue solubility coefficient of gases. To determine gas solubility in tissues of marine mammals we have adapted methods published in the human literature to study gas solubility of anaeshtetics in human and animal tissue [11-13]. Major modifications are the use of evacuated tubes to help the gas to come out of solution and to minize N_2 contamination. For this same reason, most of the work is done in a glove-box filled with pure argon.

Tissues have been collected for future determination of gas solubility in these tissues once the method has been developed and validated. Tissue from brain, heart, muscle, blubber, skin, spleen, kidney, liver, and lung are cut into small pieces, and immersed in saline solution before freezing of the samples. The tissue is then homogenized with a saline solution (12000rpm) while still cold to avoid denaturing of the proteins. The homogenate is filtered and transferred into a 15mL-evacuated tube. This is the first degasification of the tissue. The tissue is warmed up to 37°C in a water bath, and the headspace of the tube is evacuated again for further degasification of the tissue including water vapor. Following degasification, the tube is introduced in the glove box filled with argon to prevent any atmospheric contamination in the following steps. Inside the glove box, the head space is filled with N_2 (1 atm at 37°C). Tubes are placed in a thermal rocker for incubation. The time required to achieve equilibration is still to be determined. So far, samples have been left overnight to ensure that equilibration was reached. Following equilibration the homogenate alone is immediately transfered through a gas tight syringe to a new evacuated vial. This new vial is allowed to equilibrate overnight. Helium is injected to so the pressure in the the head space of the sample is 1 atm. The content of the head-space is analyzed by gas chromatography. Injection of the sample into the gas chromatograph is done using a sample loop to minimize volume injection error. We have tested our method using olive oil in order to validate the method.

RESULTS

OBJECTIVE 1

Aim 1: Obtaining morphometric data of different species. Here we present preliminary data since we need to increase the number of animals for each species studied. The size compartment of bones seems to be slightly larger in pinnipeds than in cetaceans. The size compartments of fat is larger in phocids than in cetaceans, and it is larger in cetaceans than in otariids. On the other hand, the muscle compartment is similar in cetaceans and in otariids but smaller in phocids.

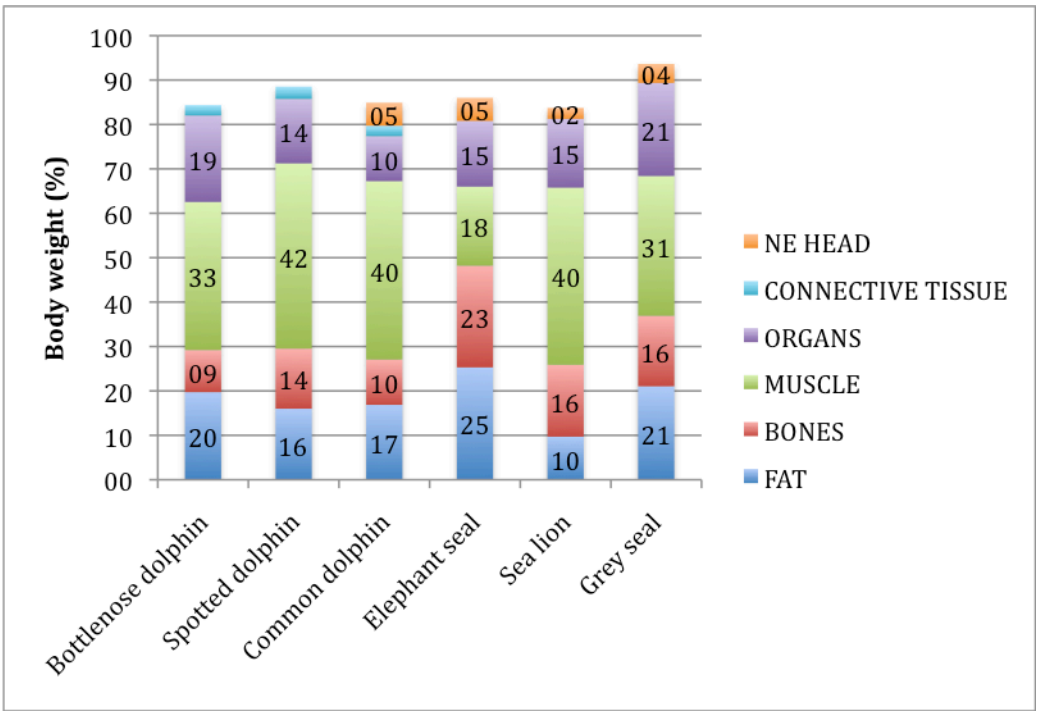

Fig. 1: Average size for compartments divided in 6 groups: connective tissue, organs, muscle, bones, fat, and "NE Head". "NE Head" represents the relative weigh of tissues of the head that were not studied (skin, fat and mucsle from the head was not studied).

In cetaceans the body compartment that changed the most with body condition was the muscle compartment:

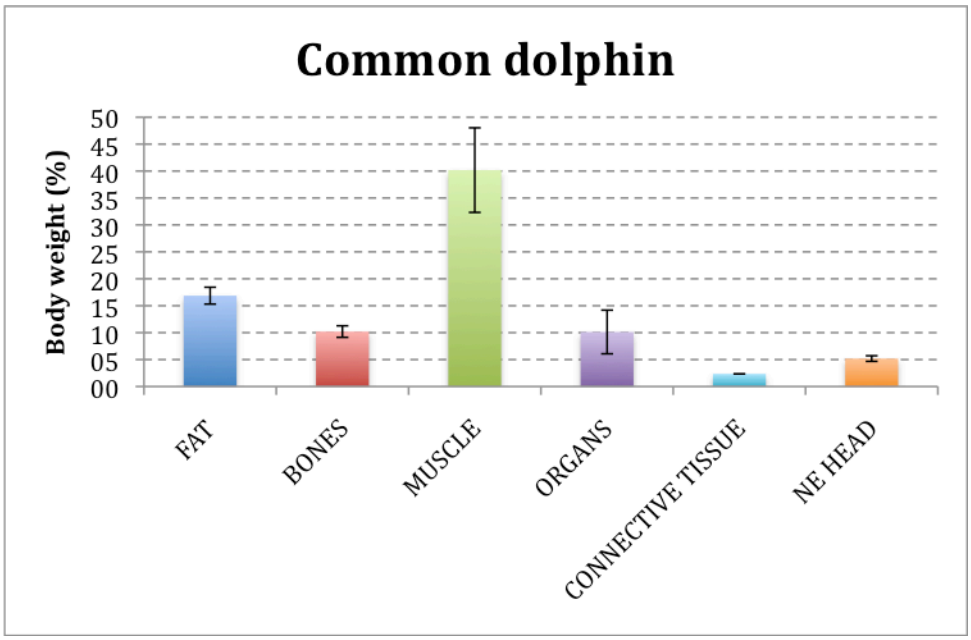

Fig. 2: Average size and standard deviation for body compartments of three common dolphins of similar age (subadults and adults) but with different body condition. Muscle was the most variable with body condition.

In contrast, the body compartment that is most affected by body condition in phocids was the fat compartment:

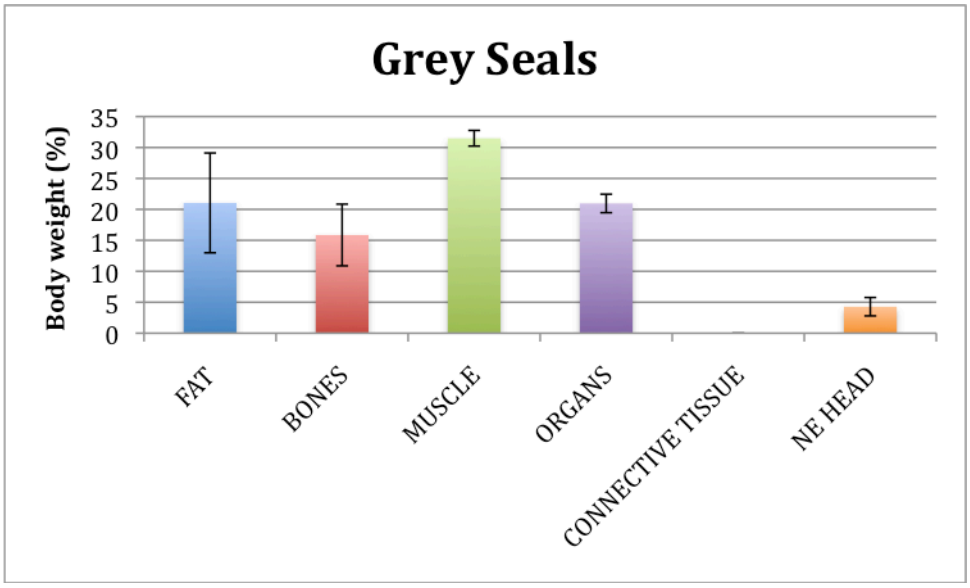

Fig. 3: Average size and standard deviation for body compartments of two grey seals similar age but with different body condition. Fat was the most variable with body condition.

As we collect more data from robust animals we will separate the data from other other animals in worst body condition which might be affecting the relative weight of the fat or the muscle compartments for modelling. On the other hand, these data is very valuable to understand better the health status of marine mammals.

OBJECTIVE 2

Aim 1: Design of anaerobic grinder.

The device was tested in three deceased specimens: a fresh animal with no bubbles, a frozen animal with no bubbles, and a fresh animal with bubbles. The method worked well in the fresh animal with no bubbles. In the frozen animal, the tissue was liquified when using the grinder. Most tissue was lost with the "water" and very little tissue came out through the needle. A similar result was obtained with the animal with bubbles. Organs were very congested and most of the tissue was lost with the blood. Based on these results, the tissue grinding device will be modified so it does not separate the blood from the tissues. This modification will allow us to determine tissue gas solubility from animals with gas bubbles. For this same reason, in order to study the gas solubility in tissues we decided to use a homogenizer instead of this device.

Fig. 4: Device for anaerobic excision and grinding of tissue

Aim 2: Determine the tissue solubility coefficient of gases. We have optimized tissue homogenization and preservation. We have found the vacuum tubes that allow us to perform the incubation in order to maximize gas exchange surface area while at the same time degassing the tissue. We have also tested control samples (blanks) and assured that the vacuum is preserved. We did four assays with olive oil to validate the methodology and obtained solubility coefficients similar to previously published. After we are certain that the method is validated with olive oil, we will do the same with saline solution and then we start working with the tissues of marine mammals.

Aim 3: Analyzing gas content in tissues. For this aim we need to improve the anaerobic grinding device. We have learnt from the solubility study that we need large sample volumes and that we might need to do the gas analyses of tissues using a GC-MS instead of a simple GC. In the solubility study we worked with saturated tissues and we are already working on the low end of the sensitivity of the detectors.

IMPACT/APPLICATIONS

Prior work has suggested that marine mammals are commonly supersaturated with gas, such that a direct ascent to the surface result in bubble formation in most tissues [14]. Recent work by Bernaldo de Quiros et al [4] has shown that gas composition analysis can discriminate between gas from decompression as opposed to decomposition. Fresh, drowned-at-depth ascended bycatch do indeed show evidence of postmortem decompression from a supersaturated state [15]. How do marine mammals normally avoid DCS symptoms when at the surface? This proposal will help improve the parameters used for modeling gas management in marine mammals and improve understanding of how these animals manage gases while diving and breathing at the surface. A better understanding of their normal physiology is required to answer this question and will help determine how they normally avoid DCS.

RELATED PROJECTS

This project is related to N000141210388 'Markers of decompression stress of mass stranded/live caught and released vs. single stranded marine mammals' where we are using a biomarker to examine bubble stress on neutrophils and endothelial cells in diving marine mammals, in collaboration with Dr Stephen Thom at the University of Maryland.

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